

Final Report

1. Title of Project: **Micromechanical Cantilever (MC) based Sensors for Chemical Species Detection**

2. Principal Investigator: Pranav Shrotriya* and Marit Nilsen-Hamilton+

* Department of Mechanical Engineering

+ Department of Biochemistry, Biophysics and Molecular Biology
Iowa State University,

3. Abstract:

Micromechanical cantilever (MC) based sensors can provide revolutionary sensitivity for forensic detection and identification of controlled substances, toxic species, biological molecules and DNA matching. Current sensor systems require extensive sample preparation and/or specialized instrumentation to identify molecules of controlled substances such as cocaine with high specificity and sensitivity. We aim to overcome these limitations by developing robust miniature sensors based on high-resolution interferometry coupled with aptamer-based receptor layers. In order to demonstrate the feasibility and forensic suitability of our approach, we will develop MC-based sensor for sensitive and specific identification of cocaine molecules.

4. Project Description:

We successfully tested a MC-based sensing approach suitable for forensic detection and identification of chemical species. The sensing strategy involves coating one surface of the cantilever with aptamer molecules that have high affinity to the analyte molecule. The presence of the target chemical species is detected by measurement of surface stress changes resulting from the formation of affinity complexes at the sensing surface without any need for labeling. Surface stress changes associated with formation of affinity complexes are resolved using high-resolution interferometric technique. We were successful in developing a differential surface stress sensor and quantifying the specificity of the cocaine aptamer to cocaine molecules.

Successful completion of this project has set the stage for development of robust sensors for detection and identification of controlled substances. We have disseminated our findings through Journal and conference publications and made presentation at MFRC annual meeting.

5. Project Objectives:

The specific aims of the project were the following:

Aim 1: Develop miniature sensor for high-resolution measurement of surface stress associated with formation of affinity complex on the sensitized surface of micro-cantilever.

Aim 2: Functionalize micro-cantilevers with an aptamer that is capable of sensitive and specific detection of cocaine molecules.

Aim 3: Characterize the sensitivity and specificity of cocaine detection with MC-based sensors functionalized with suitable receptor layers of aptamer molecules.

Results, and Discussion:

Results obtained for each aim are divided according to each specific aim:

Aim 1: Develop miniature sensor for high-resolution measurement of surface stress associated with formation of affinity complex on the sensitized surface of micro-cantilever.

We have designed and developed a differential surface stress sensor that utilizes a single-mode fiber based Mach-Zehnder interferometer for measuring cantilever deflection and consequently, the detection of chemical and biological species. The interferometric technique is amenable to miniaturization and may facilitate the integration of all components of sensors into a single microfabricated chip. Surface stress associated with alkanethiol SAM formation on gold surface is investigated to demonstrate the sensor's performance.

The differential surface stress sensor consists of two adjacent cantilevers, a sensing/reference pair, where only the sensing surface is activated for adsorption of chemical or biological molecules. Absorption/adsorption of analyte species on the sensitized surface is expected to induce differential bending and deflection between the sensing and reference cantilevers. The microcantilevers and a pair of microlens arrays are arranged in the optical arrangement shown schematically in Figure 1 to measure the differential displacement between sensing and reference cantilevers. In this optical configuration, the incident laser beams at points A and C always arrive to points B and D, respectively, regardless of their incident angle; and their differential bending produces a change in path length difference between the beams reflected from the two cantilevers.

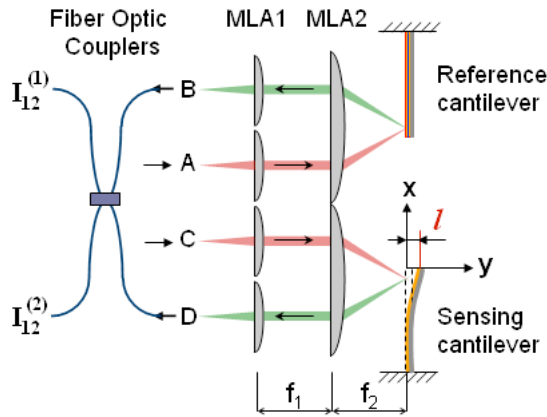


Figure 1: Schematic arrangement of the differential surface stress sensor

After reflecting from the sensing and reference surfaces, the two beams accumulate a path length difference, l , equal to twice the differential displacement between sensing and reference surface. The beams are interfered to measure the path length difference and the differential surface stress ($\Delta\sigma$) between the two cantilevers is determined using Stoney's formula.

$$\Delta\sigma = \left(\frac{E}{3(1-\nu)} \right) \left(\frac{t}{L} \right)^2 l$$

where E , is the elastic modulus and ν is the Poisson's ratio, L is the length and t is thickness of the cantilevers. Measurement of the differential surface stress ensures that detected signal is proportional to the specific absorption of analyte species on the sensing cantilever and eliminates the influence of environmental disturbances such as nonspecific adsorption, changes in pH, ionic strength, and especially the temperature

An optical circuit shown in Figure 2(A) is utilized for assembling the surface stress sensor. In the system, two adjacent rectangular-tipless AFM cantilevers were used as a sensing/reference pair. A pair of MLAs (microlens arrays) with lens of 240 μm and 900 μm diameters and pitches of 250 μm and 1mm respectively were used to direct the beams towards the sensing/reference pair. Motorized and manual actuators were used to assist in aligning of MLAs with respect to the sensing/reference

cantilevers. A bi-directional coupler was applied to split the beam from a 635nm fiber coupled laser source and delivered to MLA1 at 50/50 ratio. Other two reflected beams were interfered using the second bi-directional coupler and intensity of interfered beam was monitored using photodetectors.

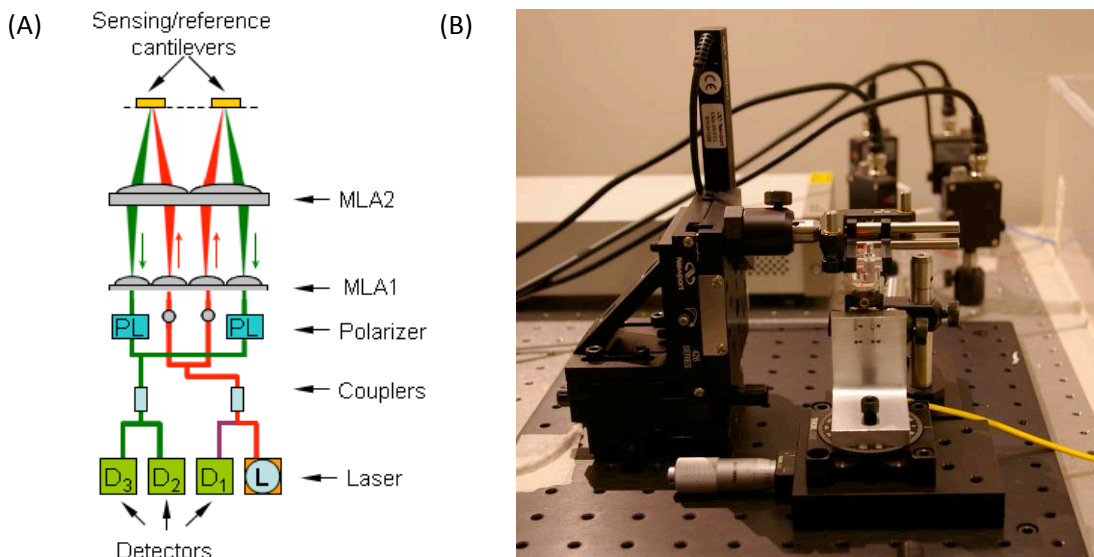


Figure 2. Optical circuit of differential surface stress sensor (A) and Photograph of experimental setup (B).

The polarization plane of the reflected beams was matched and common mode rejection was utilized to ensure maximum fringe visibility in the inferred beams. An isolation box covered all fiber couplers as well as sensor components to eliminate acoustic and vibrational noise from the system.

Silicon cantilevers used in the sensor realization are 480 μm long, 80 μm wide, and 1 μm thick with a top side coating of 5nm titanium and 30nm gold film. (Nanoworld, Switzerland). AFM cantilevers are batch productions with large variation of dimensions and mechanical properties from the manufacture's quote [9, 10]. In order to measure accurate surface stress development, the thickness of each cantilever is calculated based on the experimentally measured spring constant with the material constants [9]. Microstructure and surface roughness of the gold film were determined using contact mode AFM.

Experimental procedure

Liquid octanethiol [$\text{CH}_3(\text{CH}_2)_7\text{SH}$] was selected as alkanethiol solution and purchased from Sigma-Aldrich. AFM cantilevers were cleaned by immersing for 30 minutes in piranha solution (70% H_2SO_4 , and 30% H_2O_2 by volume) and were then rinsed in deionized water and dried in the gentle N_2 flow. Reference cantilevers were incubated in pure octanethiol solution for 12 hours to ensure the formation of a self assembled monolayer (SAM) on the gold film. Formation of a stable SAM on the reference cantilever ensures that alkanethiol molecules are only absorbed on the sensing cantilever during subsequent experiments.

Surface stress development associated with alkanethiol SAM formation was measured in three steps. In the first step, reference and sensing cantilever were mounted in the sensor and stability of the interferometer was first checked to ensure that measured signal is not affected by drift and ambient noise. In the second step, 20 mL of pure liquid octanethiol was injected into a beaker placed near the

two cantilevers. The vapors of alkanethiol solutions were confined near the cantilevers and interferometer was utilized to measure the deflection of sensing cantilever associated with deposition and formation of alkanethiol SAMs. Intensity of the interfered beams as well as laser input (I1) were monitored through Photodetectors and DAQ equipped computer. Differential surface stress, which is proportional to the cantilever deflection, is then calculated by using Stoney's Formula with obtained spring constant and geometry of the cantilever.

After the exposure to alkanethiol, both the sensing and reference cantilevers are expected to be covered with alkanethiol SAM; therefore, reintroduction of alkanethiol vapors should not cause further differential bending of the cantilevers. In the last step, sensing and reference cantilevers were again exposed to alkanethiol vapors to ensure that measured surface stress change is associated with only alkanethiol formation.

RESULTS AND DISCUSSION

Previous reports have indicated that distance of cantilever to the location where alkanethiol droplets are introduced, condition of gold surface like cleanliness and roughness, and grain structure of the gold on the cantilever's surface affect the and magnitudes of surface stress. Among those conditions, the gold morphology of the cantilever dominantly influences the development of the surface stress during the formation of Alkanethiol SAMs [4]. measurement and determined grain size was 40 ± 10 nm with the mean square roughness of 2.07 ± 0.23 nm for the 750 nm scan size. Surface stress is mainly induced high affinity of sulfur atom in the thiol head to gold atom of cantilever surface, structural phase transitions, and molecular interaction between chains while developing in self-assembled monolayers. During SAM formation, it is undergone tensile first and instantaneously developed with the compressive stress due to bimetallic effect.

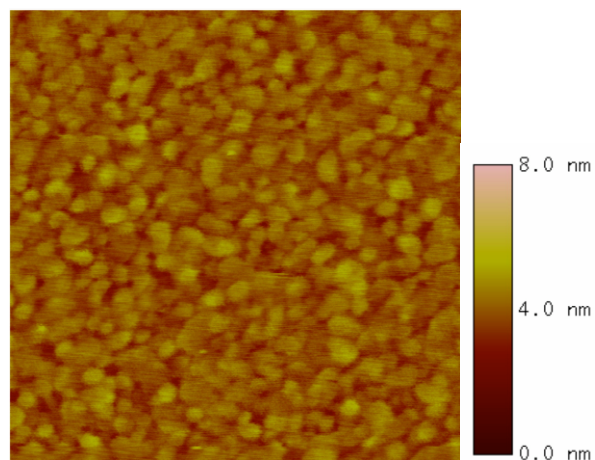


Figure 3. AFM image (750nm×750nm) of grain structure of gold film on AFM cantilevers

The differential bending and the corresponding surface stress change during formation of alkanethiol SAMs on the sensing cantilever respect to reference cantilever are shown in Figure 4(A). In order to verify those changes are only due to the absorption of alkanethiol molecules on gold surface, the nominal drifts before and after injection were also calculated and compared in Figure 5(B). As soon as alkanethiol solution was injected, the sensing cantilever undergoes tensile stress first and compressive surface stress develops subsequently. According to Figure 4(A), alkanethiol SAMs and associated stress took approximately one hour to reach saturation. Surface condition of cantilevers such as cleanliness, roughness, and the condition of gold deposition on the cantilever's surface influence on the pattern as well as magnitude of surface stress change. In addition, the distance of cantilever to the location where alkanethiol droplets are introduced was 10 cm away. The second development of surface stress change in Figure 5(A) is due to either uneven surface condition of cantilever or time difference in arriving target molecules (Sulfur atoms) on sensing cantilever. As a result, final surface stress change was 0.28 ± 0.02 N/m and the corresponding differential banding was 180 ± 10 nm at grain size of gold surface was 40 ± 10 nm.

Plots taken before and after the SAM formation on the sensing cantilever show that surface stress change was within the system's normal noise range, ± 0.02 N/m. Especially, a minimal surface stress change during re-introduction of the alkanethiol vapors indicates that both sensing and reference cantilever are covered with alkanethiol SAM. Furthermore, it indicates surface stress change observed during the first introduction is unambiguously associated with SAM formation on sensing cantilever.

Aim 2: Functionalize micro-cantilevers with an aptamer that is capable of sensitive and specific detection of cocaine molecules. We have validated the functionality of the aptamer selected for this investigation through the following assays:

- Dye displacement assay
- Fluorescence resonance energy transfer assay (FRET)

The results of this assays indicated that the cocaine aptamer reported in literature has a lower sensitivity for binding with its analyte (cocaine) in comparison to other known aptamers. In order to guide the experimental work and future development of highly sensitive detection, the isothermal titration calorimeter was utilized for quantification of the binding between cocaine aptamer and cocaine molecules.

Dye Displacement Assays: Dye displacement assay were used to determine the efficacy of the cocaine aptamer for binding with cocaine molecules. In the displacement assays, the aptamer molecules initially bind with the dye and on the introduction of cocaine, dye is displaced from the

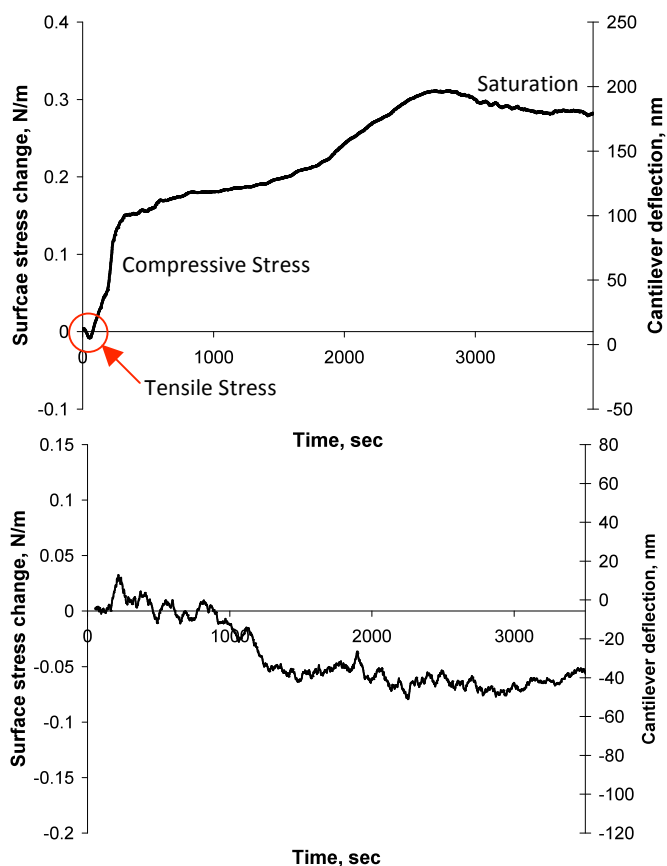
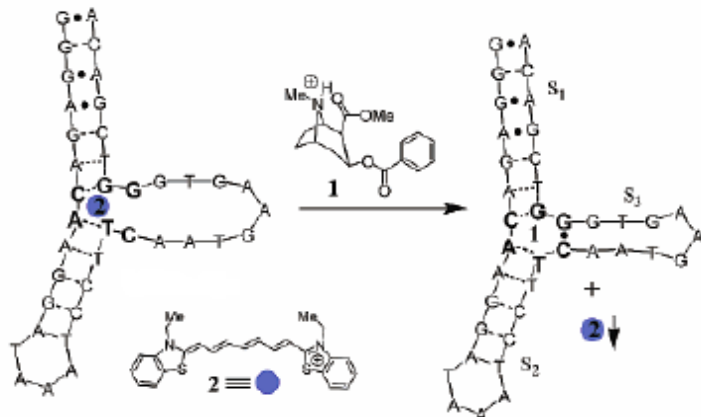


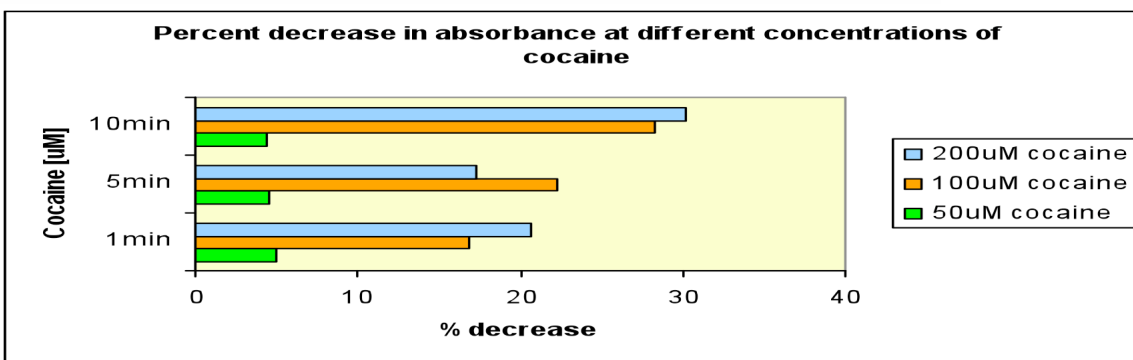
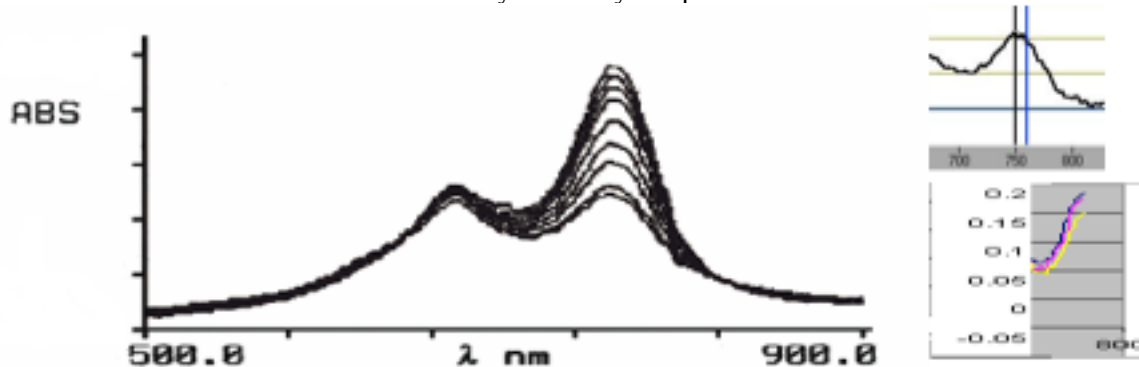
Figure 4. Surface stress change and the corresponding sensing cantilever deflection respect to reference cantilever (A) Differential surface stress during deposition; (B) Differential surface stress due to alkanethiol exposure after deposition.

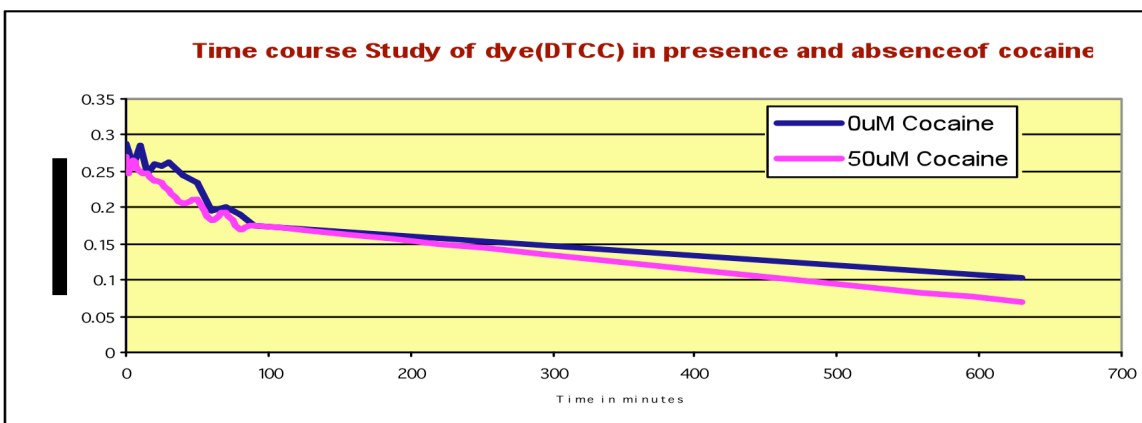
aptamers as schematically represented below:



Aptamer complexes with the dye and its displacement with cocaine leading to change in absorbance

The dye molecule used was 3,3'-Diethylthiatricarbocyanine iodide. Attenuation of absorbance and eventual precipitation of the dye on introduction of cocaine molecules in the aptamer dye solution was monitored. Representative experimental results of the colorimetric readout at 760 nm, percent decrease in absorbance and the time history of the dye in presence of cocaine are shown below:

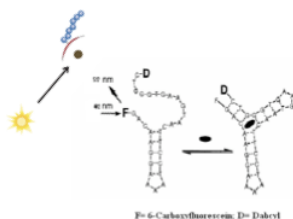




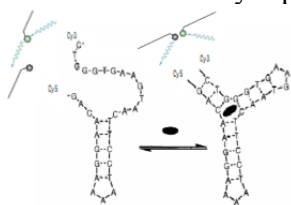
These results indicate that ability of aptamer to undergo visible changes upon recognition of their ligands in "mix and measure" assays could result in simpler and less expensive colorimetric analytical procedures, including spot tests for small molecules. However, the cocaine aptamer does not have a stable response to introduction of cocaine molecules.

Fluorescence resonance energy transfer assay (FRET assay): Two different modifications in the structure of the cocaine were investigated for this assay:

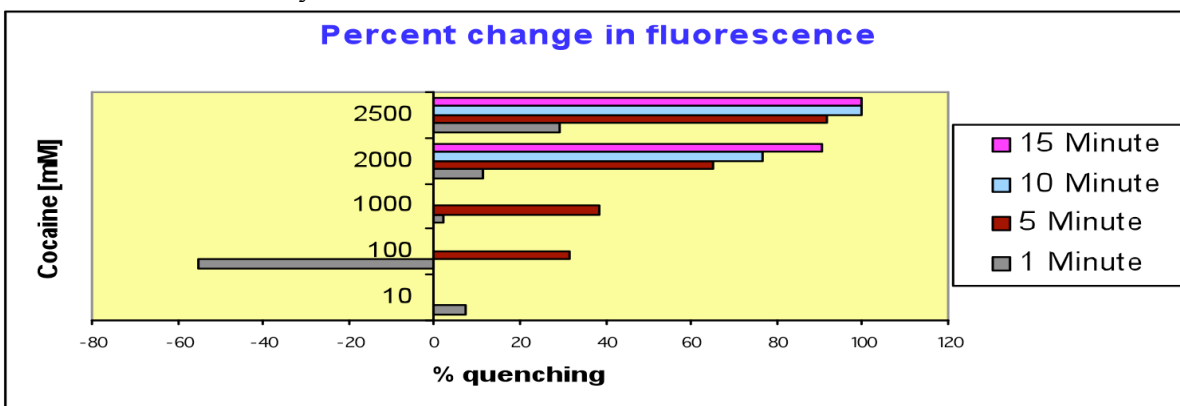
- In the first set of experiments, aptamer was modified such that unbound molecules fluoresce and binding of the aptamer with cocaine leads to quenching of the fluorescent radiations. 6-Fluorescein: (λ_{em} 490nm λ_{ex} 514nm, was used as the fluorescing molecule and Dabcyl was used as the quencher for the emitted radiations. Details of the structure change and the principle of quenching are shown below:



- In the second modification, aptamer structure was modified such that the emitted fluorescent radiation is shifted in wavelength on the binding with cocaine molecule. Cy3 (550/564nm) and Cy5 (648/668) were used because of the overlap in their emission and absorption spectra. On binding with cocaine molecules, radiation emitted from Cy3 would be absorbed by Cy5 leading to a shift in emitted radiation. The structural change and principle of FRET are schematically represented below:

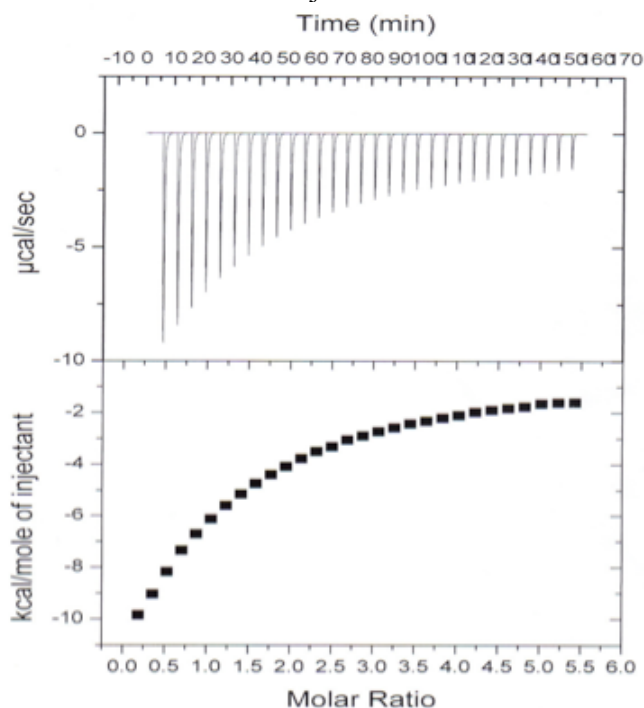


Results of the FRET assay are summarized in the chart below:



Results of both dye displacement and FRET assay indicate that sensitivity of the cocaine aptamer for cocaine molecules is low compared to other aptamer/analyte combinations ($K_d \sim \text{nm}$). These results suggest the need for quantification of the disassociation constant in order to determine the sensitivity and efficacy of the cocaine aptamer for detection of cocaine molecules.

Isothermal Titration Calorimetry (ITC) experiments: In these experiment, the heat evolved or absorbed in liquid samples as a result of mixing precise amounts of reactants was measured to determine the proportion of binding between cocaine aptamer and cocaine molecules. A representative data set from the ITC experiment on cocaine aptamer and cocaine molecule that shows the heat released at each injection of cocaine molecules in cocaine aptamer is shown below:



The analysis of these results indicate that the binding between cocaine aptamer and cocaine molecules can be quantified as follows:

$$N = 1.07 \pm 0.221$$

$$K = 3.83E3 \pm 561$$

$$\Delta H = -2.991 \text{E}4 \pm 7324$$

$$\Delta S = -83.9$$

Final concentration of Aptamer: 100 μ M

Final concentration of Cocaine: 500 μ M

$$K_d = 261 \mu\text{M}$$

“N”= Average number of binding sites per mole of aptamer

These results clearly indicate that the present cocaine aptamer has moderate sensitivity to cocaine molecules.

Aim 3: Characterize the sensitivity and specificity of cocaine detection with MC-based sensors functionalized with suitable receptor layers of aptamer molecules.

Further work is continuing to complete this aim. At the end of the project duration, we did not have any experimental results to report on this aim.

CONCLUSIONS

High resolution ($\pm 0.02 \text{N/m}$) of surface stress development associated with the formation of alkanethiol self-assembled monolayers on a gold surface in vapor phase was measured to characterize the response of the sensor. Among several critical factors in developing surface stress change induced by formation of alkanethiol SAMs, grain structure of gold surface and distance between droplets and cantilevers are identified. The experimental results confirm that the sensitivity of sensor measurement is not dependent on the distance between the sensing surface and detectors; thus, surface stress sensor is amenable for miniaturization and array of sensors would be easily fabricated on a single MEMS device.

Binding of cocaine aptamer and cocaine molecule was validated with dye displacement and FRET assays. ITC experiments were used to quantify the disassociation constant and stoichiometry of the reaction between cocaine aptamer and cocaine molecules.

Further work is required to characterize the sensitivity and specificity of cocaine detection with MC-based sensors

6. Dissemination Discussion: Results of our work have been disseminated through four conference presentations (Conferences of Toxicology Society, Material Research Society, SPIE and American Society of Mechanical Engineers) to the academic community in diverse disciplines. We have also made a presentation of our results at the MFRC annual director's meeting in Bemidji, MN to disseminate our findings in the forensic community. This work has partially contributed to one journal publication in Applied Physics Letters (APL).
7. Appendix.
 - a. Discussion of problems that have arisen.

None, however, there is a need for further work to characterize the sensitivity and specificity of cocaine detection with MC-based sensors